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UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 289

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER September 21, 1915

RED-CLOVER SEED PRODUCTION:

POLLINATION STUDIES

By

J. M. WESTGATE, Agronomist, and H. S. COE, Scientific Assistant, Office of Forage-Crop Investigations, in Collaboration with A. T. WIANCKO and F. E. ROBBINS, of the Indiana Agricultural Experiment Station, and H. D. HUGHES, L. H. PAMMEL, and J. N. MARTIN, of the Iowa Agricultural Experiment Station

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INTRODUCTION.

For a number of years the quantity of seed of red clover (*Trifolium pratense*) produced in this country has been insufficient to supply the demand for reseeding purposes in the clover-belt States. This not only has caused the seed to be high in price, but has resulted in the importation of large quantities of foreign seed, some of which, on account of the impurities present and its low vitality, has been considerably less desirable than the ordinary home-grown strains.

The prime importance of clover in the ordinary farm rotations in the corn and clover belt States makes the continued maintenance of the clover acreage of great moment to the agricultural prosperity of the country. This problem has been approached from four different angles. First, to determine the minimum amount of seed necessary to obtain a stand, so that much less than the quantity of seed ordinarily sown will be sufficient to produce a satisfactory yield, for any

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reduction in the quantity of seed required to sow an acre will proportionately increase the acreage throughout the country which can be sown with the available supply of seed. The second line of attack has been to determine the environmental conditions necessary to the maintenance of a satisfactory stand of clover. That these conditions are less favorable than they have been in the past is indicated by the increasing difficulty experienced by many farmers in maintaining clover in the ordinary rotations. A third line of attack has been the possibility of developing a heavy-seeding, hardy strain of clover with good forage and hay producing qualities. The fourth line of attack, the one with which the present publication is concerned, has been a study of various means of affecting the yield of clover seed under field conditions as they exist throughout the clover-growing sections of the country. One phase of this work has been to determine the effect of the time of cutting or clipping the first growth on the seed production of the subsequent crop. The second phase, that with which this bulletin is primarily concerned, has been the effect of various mechanical forms of pollination upon the quantity of seed produced.

PREVIOUS INVESTIGATIONS ON THE POLLINATION OF RED CLOVER.

Since the time of the publication of the statement by Darwin (6, p. 361; 7, p. 90)[1] that 100 heads of red clover on plants protected from insects during the blooming period did not produce a single seed while a similar number of heads exposed to insects produced an average of 27 seeds per head, many scientists have investigated this subject. Knuth (22, v. 1, p. 36–37; v. 2, p. 289) accepts Darwin's experimental results and states that red clover, crimson clover, and white clover are among the best examples of self-sterility in plants. Stebler and Schröter (39, p. 123) in discussing the pollination of red clover say that there is no experimental evidence to show that pollen from a flower can not, when applied to its own stigma, fertilize the ovules, but they also state that pollen which is effective in producing fertilization has in all probability come from some other flower. The same authors (40, p. 14, 122) in a later edition state that red clover is self-sterile. Frandsen, according to Lindhard (23), found red clover to be practically self-sterile. From 1,235 flowers in 1910 and 1,305 flowers in 1911, which were self-pollinated by Frandsen, 0.07 per cent set seed. In 1910 Frandsen pollinated 1,488 flowers and in 1911, 1,455 flowers with pollen from other heads on the same plants; 0.8 per cent of the flowers set seed in 1910 and 0.4 per cent in 1911.

Wallace (43, p. 121) states that insects must perform the indispensable work of cross-pollinating red clover, but later says (44) that he has been inclined to think that climatic conditions rather than the

[1] Reference is made by number to "Literature cited," p. 29.

presence or absence of insects influence seed production. The work of Sirrine (37, p. 89–90), as well as that of Witte (46), showed red clover to be self-sterile. In the experiments of Cook (5), Shamel (36), and Kirchner (21) no seed was produced when heads were covered before blooming and not pollinated. Fruwirth (11; 12, p. 163–166) did not obtain a single seed when heads under cover were left undisturbed or when they were pollinated with pollen from another head on the same plant, while heads pollinated with pollen from another plant produced seed. Bolley (4) obtained but two seeds from one head of a large area which was placed under a fine screen before any of the flowers came into bloom. He states that insects other than bumblebees must pollinate the flowers, since the bumblebees were scarce and the clover set well. Genevier (13) states that the fertilization of clover does not depend on the presence of bumblebees. Pammel and King (32) report but two seeds from 643 heads which were allowed to mature under a screen cover, while Washburn (45) says that only by the aid of bumblebees was he able to obtain seed.

Armstrong (1), in writing about New Zealand, says there is every reason to believe that numerous individuals belonging to *Trifolium pratense* are self-fertile and that they produce self-fertile progeny. According to him the American strain is usually, if not always, self-fertile. McAlpine (24) discusses Garton's experiments, which show that the self-fertilizing property is as common with red clover as it is with the bean. The following is quoted from Kerner (20, p. 407): "Pisum and Ervum, Lotus and Melilotus, the various species of Trifolium, almost all of them, when unvisited by insects, ripen seed, only a few species here and there being infertile when dependent upon their own resources." Nothing definite can be taken from Kerner's statement, since he does not quote any species or give definite exceptions to his statement. Hopkins (14) says he is not ready to admit that self-fertilization does not take place and that he is inclined to believe a crop of seed can be grown without the aid of bumblebees. The same author (15, p. 73) states that honeybees serve the same purpose as bumblebees in cross-fertilizing red clover. The work of Beal (2, p. 325–328) shows that bumblebees increased the seed production about four times, since in a check cage he received 25 seeds from 50 heads, while in the cage where bumblebees were placed 94 seeds from 50 heads were obtained. Martinet (27) found red clover to be self-fertile, stating that cross-pollination might have been brought about by very small insects (undoubtedly meaning thrips). Fruwirth (12, p. 163–166), however, showed that thrips transferred from other clover fields in large numbers produced no seed in his experiments. Meehan (28) states that a careful examination of the clover flower in all its stages convinced him that from its structure and behavior it was self-fertile. It is still an open

question whether or not red clover is self-fertile, according to Smith (38, p. 236).

Garton, according to Wallace (44), claims that red-clover flowers are cleistogamous, but Martin (26) in his work on the cytology of red-clover flowers disproved this theory. Garton attempts to prove that the flowers are cleistogamous by saying that the ovules are well formed by the time the flowers open. The ovary is quite large at this time, and it was undoubtedly taken to be a developing ovule. Pammel and King (32) record that self-fertilization was accomplished in some experiments at Ames, Iowa, by irritating the stigmas. Hunt (19) speaks as follows: "It has long been recognized that red-clover and other leguminous flowers may be self-pollinated, although it has never been determined whether self-pollination or cross-pollination most commonly occurs."

According to Dunning (8), after the introduction and establishment of bumblebees in New Zealand red clover seeded abundantly, but previous to this time he says it seeded very little. The Agricultural Gazette of New South Wales (9, 16) maintains that bumblebees were introduced into New South Wales from New Zealand so that they would be able to produce clover seed for home use, which up to this time was largely imported. At Failford, New South Wales, red clover seeded abundantly (35), although no bumblebees had been noticed in that vicinity. The pollinating was thought to have been done by several native insects. This was several years after the introduction of bumblebees. Later (17) it was stated that bumblebees had become well established.

Waldron (41, 42) found in his experiments that bumblebees were responsible for about 95 per cent of red-clover seed and that a small quantity may be produced by natural self-pollination.

Müller (29, p. 184–186) states that when a bee draws its proboscis out of a clover flower cross-pollination is assured and self-pollination may also take place, but that the self-pollination is probably neutralized and superseded by the immediately preceding cross-pollination.

Folsom (10, p. 116) considers the Italian race of honeybees as important as the bumblebees in clover-seed production, while Armstrong (1) claims that honeybees are able to extract nectar from red-clover flowers in New Zealand. Pammel (31, p. 172) shows that honeybees are able to collect pollen from red-clover flowers and thereby cross-pollinate them. Robertson (33, p. 177) states as follows: "But while butterflies may sometimes effect cross-fertilization of the red clover, they are of doubtful value, if not injurious. * * * But butterflies can insert their thin tongues without depressing the keel, and, even if they get a little pollen on their thin proboscides, it is apt to be wiped off by the closely approximated tips of the petals, which close the mouths of the flowers."

OUTLINE OF POLLINATING EXPERIMENTS.

It is a well-known fact that the yield of clover seed varies greatly from year to year, and no distinct correlation with any marked climatic factors has been determined. It was thought that possibly the absence of suitable pollinating insects, such as bumblebees, might in some seasons be responsible for the reduced yields of seeds. This is especially true when conditions were such that there was no other apparent reason for the failure of the crop to set seed. In order to obtain light on this point, a series of experiments was outlined to determine (1) whether clover flowers were able to set seed without the assistance of outside agencies; (2) whether clover flowers were able to set seed when their own pollen was transferred to their stigmas by outside agencies; and (3) the relative efficiency of the honeybee and the bumblebee as cross-pollinators of red clover.

In order to overcome any local environmental factors, the experiments were conducted at Ames, Iowa, and La Fayette, Ind., and were repeated to some extent at the Arlington Experiment Farm, Va. The work on individual clover heads was performed on heads protected from the action of insects by tarlatan cloth. This cloth has about twice as many meshes to the linear inch as ordinary mosquito netting. Where numerous plants were to be protected from all outside agencies, cages of wire screen having 14 meshes to the linear inch were used. In some instances, where it was desired to permit the entrance of all insects smaller than bumblebees, cages made of galvanized-wire screen having four meshes to the linear inch were employed.

All work was done on second-crop red clover unless otherwise specifically stated.

STRUCTURE OF THE RED-CLOVER FLOWER.

The heads of red clover contain from 35 to 150 flowers each, and according to Pammel and Clark (30) the average number per head for black loam soil at Ames, Iowa, is 71.1 for the first crop and 98.1 for the second crop

The flowers of red clover consist of a green pubescent calyx with five pointed lobes and an irregular magenta or purple corolla of five petals (Fig 1.) The claws of the petals are more or less united to the staminal tube This staminal tube is formed by the union of the filaments of the nine inferior stamens To the greater portion of the anterior end of this common tube, formed by the uniting of the claws of the petals with the staminal tube, is attached the broad base of the vexillum. The carina, which is composed of two petals united at one edge, is attached to the inferior part of the edge of the tube

not occupied by the vexillum. Even though the base of the carina is narrow it is able to return to its normal position shortly after being bent downward. The alæ are attached by their flexible claws to the common tube. Before a flower opens the alæ are pressed closely to the carina, although as the flower matures they spread apart. The staminal tube splits superiorly to admit the tenth free stamen. The filament of this superior stamen lies along the side of the staminal tube and therefore does not interfere with the proboscis of a bee which is inserted to collect nectar. Nectar is secreted at the bases of the stamens and accumulates in the staminal tube around the base

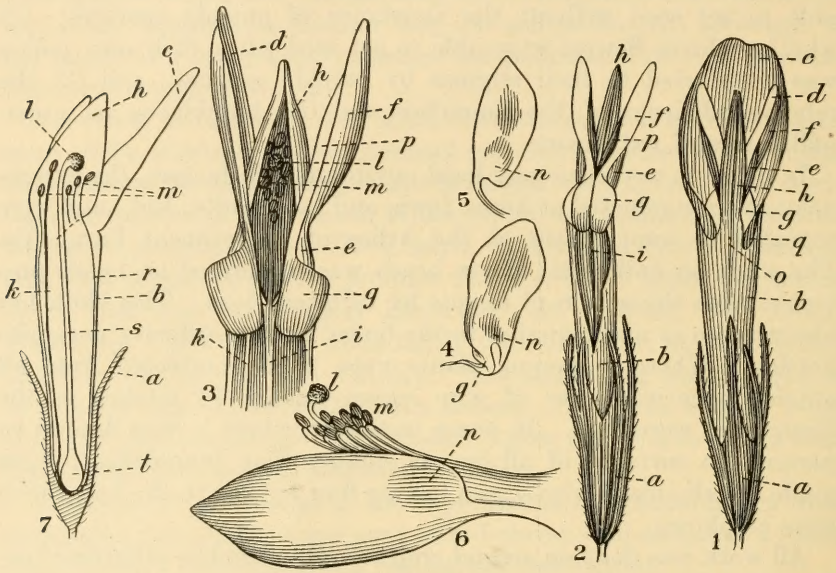

FIG. 1.—Different parts of a red-clover flower: 1, Anterior view of flower; 2, posterior view of flower after the vexillum has been removed; 3, posterior view of flower, showing the carina, which has been forced apart (twice the magnification of the other drawings); 4, right ala, from within; 5, right half of carina, from without, the claws of 4 and 5 having been partly broken off; 6, the essential organs emerging from the depressed carina; 7, longitudinal section of a flower. *a*, Calyx; *b*, tube formed by the partial union of 9 filaments with the claws of the vexillum, alæ, and carina; *c*, vexillum; *d*, concave part of the inner side of ala; *e*, lower border of ala, bent outward; *f*, outward surface of ala; *g*, pouched swelling on the base of an ala; *h*, carina; *i*, style; *k*, superior free stamen; *l*, stigma; *m*, anthers; *n*, point of union between alæ and carina; *o*, point of flexure of the carina; *p*, part of the upper border of ala, bent outward; *q* downward extension of the vexillum; *r*, staminal tube; *s*, style; *t*, ovary. (After Müller in part.)

of the ovary. The filaments which compose the staminal tube separate in the hollow of the carina. Each filament bears a fertile anther. The pistil is inclosed within the staminal tube, the upper part of the style and stigma of which are inclosed with the anthers in the carina. The stigma is situated slightly above the stamens in most flowers, although in some the anther of the longest stamen is as high as the stigma.

When a bee inserts its proboscis into the staminal tube, it is inserted between the vexillum and the carina. In doing this the carina and

alæ are pressed downward and the stigma and anthers are thrust up against the bee's head. Since the carina and stamens are elastic, the pollen is thrown with considerable force against the head of the bee. When the bee releases the pressure on the carina and alæ, the parts return to their normal position on account of the elasticity of the base of the carina and a small dilated vesicular process at the base of each ala. (Fig. 1.)

LENGTH OF THE COROLLA TUBE OF RED-CLOVER FLOWERS.

The corolla tube of red clover is stated by Knuth (22, v. 2, p. 289) and Müller (29, pp. 184–186) to be from 9 to 10 millimeters in length. Pammel and King (32) report an average length of 9.4 millimeters for 450 flowers. Schachinger, according to Fruwirth (12, pp. 163–166), says the corolla tubes are shorter in the second crop than in the first crop, and for this reason smaller bees are able to work on the second crop than on the first.

Fifteen corolla tubes from each of 28 heads of first-crop red clover were measured at Ames, Iowa. The greatest variation found in different flowers of the same head was 2 millimeters. The 420 corolla tubes varied from 8.5 millimeters to 11.5 millimeters, with an average length of 9.6 millimeters.

DEVELOPMENT OF THE FLOWERS OF RED CLOVER.

The stamens of red clover develop much more rapidly than the pistil, and the length of the longer set exceeds that of the pistil until near the time the flower opens. The pollen is formed in the longer stamens through the division of the mother cells when the pistil is about 0.25 millimeter in length. The division in the pollen mother cells of the shorter stamens closely follows that in the longer stamens. When the pistil is about 1 millimeter in length, only about one-twelfth of its length at maturity (fig. 2, *A*), the pollen grains are apparently mature so far as their size, their shape, and the thickness of their walls are concerned. At this stage the two ovules are well formed, but the egg and endosperm cells are not developed till later and are not ready for fertilization until just previous to the opening of the corolla. The later development of the pollen consists in protoplasmic changes. After the pollen grains have reached their mature size and their walls have become mature the protoplasm shows very little or no granular nature. Just before the flowers open the protoplasm becomes very dense. At this stage the protoplasm contains much oil in the form of an emulsion. The pollen will now germinate.

The pistil has a stylar canal reaching from the ovary almost to the stigma. Just previous to and during the opening of the corolla the pistil elongates more rapidly than the stamens, and as a result the stigma is usually pushed beyond the anthers in the open flower

(fig. 2, *B*). The stigmatic surface is papillate and has a fringed appearance in the mature flower. The papillæ contain much oil and have rather heavy walls, which react to the test for cutin.

Both ovules develop embryo sacs (fig. 2, *C*). Fertilization usually takes place in each ovule; but only one, so far as observed, matures into a seed. Should plants occur that mature both ovules, there would be an opportunity to produce strains with twice the seed-yielding capacity of those now grown.

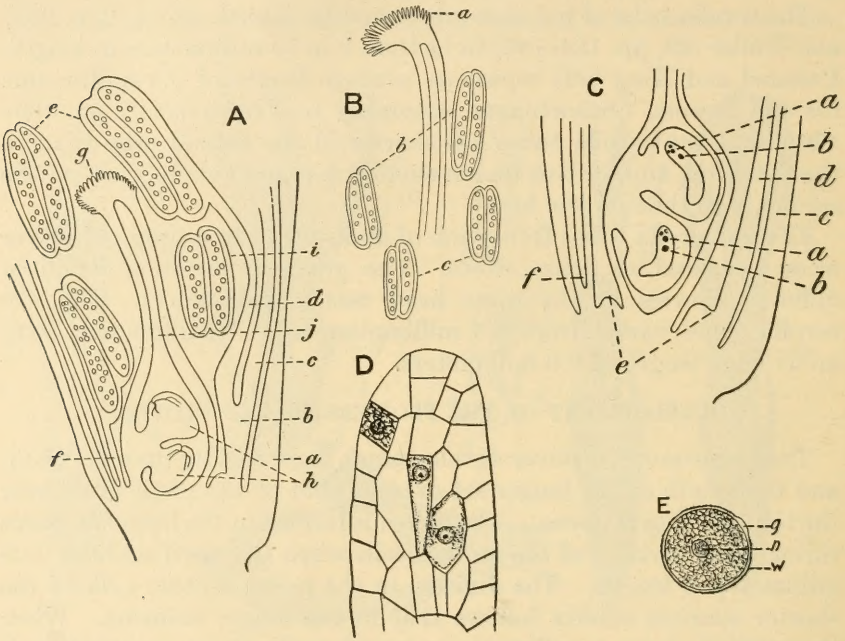

FIG. 2.—Red-clover flowers, showing different stages of development. A.—Lengthwise section of a red-clover flower at an early stage (×50): *a*, Calyx tube; *b*, staminal tube; *c*, standard; *d*, one of the long stamens; *e*, anthers of two long stamens; *f*, free stamen; *g*, stigma; *h*, the two ovules; *i*, anther of a short stamen; *j*, stylar canal. B.—Lengthwise section of an open flower, showing the character of the stigma and its position relative to the anthers (×25): *a*, Stigma; *b*, anthers of two long stamens; *c*, anthers of two short stamens. C.—Lengthwise section through the base of a flower, open and ready for fertilization (×40): *a*, Egg; *b*, endosperm cell; *c*, calyx; *d*, staminal tube; *e*, nectar glands; *f*, free stamen. D.—A median, longitudinal section through the nucellus of a sterile ovule which should have been ready for fertilization, the flower being open; all cells remained vegetative and no reproductive cells were produced (×18). E.—Pollen grain (×325): *g*, Germ pore; *n*, nucleus; *w*, wall.

INFERTILE OVULES OF RED CLOVER.

Infertile ovules are a common occurrence in red clover and occur to a considerable extent throughout the season. A section through the nucellus of an infertile ovule is shown in figure 2, *D*. In the infertile ovules all cells remain vegetative and no embryo sac is formed. The largest percentage of infertility has been found to occur in first-crop red clover, and this infertility appears to accompany moist soil and atmospheric conditions. During the first crop many plants produce

100 per cent of infertile ovules. With such plants the presence of bees is not a matter of importance, for the ovules have no reproductive cells; hence there can be no fertilization and no production of seed. During the second crop, when the season is generally dry and favorable for seed setting, there is some infertility, ranging from a low percentage or none in some plants to a high percentage in others. It is very probable that this infertility of ovules is to a greater or less degree a hereditary character and that the production of a high-yielding strain will consist, among other features, in selecting those plants with the least tendency toward infertility.

POLLEN OF RED CLOVER.

The pollen grains of red clover are almost globular when turgid, with a little flattening at the germ pores. When measured in a 25 per cent cane-sugar solution the pollen grains have an average size of 44.5 by 43 μ (fig. 2, *E*). The grains are not fully turgid when shed from the anthers and one diameter in each is shortened and the other diameter lengthened by an infolding of the wall. In this condition Martin (25) found the average dimensions of 100 pollen grains to be 26 by 48 μ, while Miss Clark (30) found the average size of 1,024 pollen grains to be 31.7 by 56.29 μ.

When dropped in water the pollen grains take it up very rapidly and burst almost instantly. On account of this feature of the pollen there can be little effective pollination when the flowers are wet. Pollination at night or in the morning when the flowers are wet with dew is not likely to be effective.

Germination of the pollen of red clover was found by Martin (25) to depend upon a proper water supply. Good artificial germination can be secured on parchment paper or animal membranes which are just moist enough to permit the pollen to absorb the requisite amount of water for germination. Germination takes place within a limited range of variation in the water supply, and it is only by trials of wetting and drying that the proper moisture content of the membranes may be secured. Under proper conditions of moisture and temperature, germination takes place usually in 8 to 10 minutes.

FUNCTION OF THE STIGMAS OF RED-CLOVER FLOWERS.

Microchemical tests of the stigmas of red-clover flowers show no sugars or starches present. An oily emulsion, however, does occur in the papillæ. Crushed stigmas placed on animal membranes had no apparent effect on the germination of the pollen or on the directions of the tubes.

When pollen is deposited on the stigmas it lodges between the papillæ, takes up water, and soon becomes turgid, but the water supply is so regulated by the stigmas that no bursting occurs. It

is probable that the only function of the stigmas in the germination of the pollen is that of supplying the requisite amount of moisture to the pollen. If such is the function of the stigmas, a wet soil or humid atmosphere, both of which tend to increase the water content of the stigmas, may allow the pollen to absorb too much water and thus prevent fertilization. Martin (25) found pollen lying dormant on stigmas 18 hours after pollination during cool, moist weather. This dormancy might have been due to the effect of low temperature upon the pollen alone, but could have been due to an interference with the moisture adjustment.

FERTILIZATION OF RED-CLOVER FLOWERS.

TIME REQUIRED BETWEEN POLLINATION AND FERTILIZATION.

The time between the pollination and the fertilization of red-clover flowers varies. Flowers pollinated in July, when the temperature was high and killed 18 hours after pollination, showed that in most cases fertilization had taken place. In October, when the temperature was much lower, the time between pollination and fertilization ranged from 35 to 50 hours.

NECESSITY OF FERTILIZATION OF RED-CLOVER FLOWERS.

It has been reported that red clover is able to develop seed without fertilization; but field experiments, as well as laboratory tests, have disproved this statement. One of the most noticeable features of this work was the fact that all the flowers of heads which were covered with tarlatan before they came into bloom and left in this condition until they withered remained in full bloom from 9 to 10 days. Flowers of red clover wither shortly after fertilization takes place. This is why red clover heads usually contain flowers in bud, in bloom, and withered at the same time.

In order to further test the necessity of fertilization, a large number of heads were covered with tarlatan before any flowers came into bloom. An examination of more than 500 flowers at various times after they began to wilt showed no embryos. The ovules were disintegrating.

POTENCY OF POLLEN IN SELF-POLLINATION.

In order to determine the potency of pollen in self-pollinated flowers of red clover, a number of heads were covered with tarlatan two or three days previous to the opening of the flowers. Some of these covered flowers were self-pollinated by springing the carinas, while the rest were cross-pollinated by springing the carinas and applying pollen from flowers on other plants to their stigmas. By mounting the pistils of these flowers in a 30 per cent sucrose solution

and flattening them with a little pressure on the cover glass, the pollen tubes could be traced through the stylar canals, as pollen tubes have a denser and more granular content than the cells of the style.

An examination of 30 flowers which had been self-pollinated for 55 hours showed good germination on the stigmas but no fertilization. The number of pollen grains germinating on the stigmas ranged from 3 to 25 in each of the 30 flowers. The tubes had made a slow growth and none exceeded 4 millimeters in length. An examination of 20 flowers which had been self-pollinated for 90 hours showed that one pollen tube had attained a length of 7.5 millimeters, while the others were 5 millimeters or less in length. At this rate of growth the longest tube would have required about 48 hours more to reach the ovules, or about six days to traverse the entire distance from stigma to ovule. Flowers examined four days after springing the carinas showed the eggs in a disintegrated condition. It is therefore probable that in case of self-pollination the pollen tubes do not reach the ovules in time to effect fertilization.

An examination of the 30 flowers which had been cross-pollinated for 55 hours showed that fertilization had taken place in all of them.

CROSS-POLLINATION AND SELF-POLLINATION OF RED CLOVER.

The results published by previous investigators on the cross-pollination and the self-pollination of red clover do not agree. These investigators appear to be about equally divided as to whether red-clover flowers are self-fertile or not. The experiments of Frandsen, according to Lindhard (23), Fruwirth (12, p. 163–166), and others show that red-clover heads which were covered during their blooming period and not pollinated failed to set seed Frandsen and Fruwirth also show that pollen must come from an entirely separate plant in order to fertilize the ovules of red-clover flowers. On the other hand, Garton, according to McAlpine (24) states that self-pollination is as common with red clover as it is with the bean.

The relative efficiency of the bumblebee and honeybee as cross-pollinators of red clover has also been discussed by scientific investigators, as well as by agricultural papers and bee keepers. Bee men generally agree that the Italian race of honeybees can extract nectar from red-clover flowers. Little has been said, however, about the ability of the honeybee to collect pollen from red clover.

In view of the above diverse opinions in regard to the self-pollination and the cross-pollination of red clover, a number of experiments were outlined in order to determine (1) whether red-clover flowers were self-fertile; (2) if self-fertile, whether any effective method of self-pollination could be found which would be applicable for use on a field scale; and (3) the relative efficiency of the bumblebee and honeybee as cross-pollinators of red clover.

ARTIFICIAL MANIPULATION OF CLOVER HEADS.

Experiments were conducted to determine, if possible, the effect on seed production of various types of artificial manipulation of the clover heads while the flowers were in bloom (fig. 3). A sufficient number of heads were selected on each plant so that the work could be conducted on heads covered with tarlatan (fig. 4) and on heads exposed to the action of insects. The experiments on the heads exposed to the action of insects were to determine whether the artificial manipulation of the flowers would have any harmful effect on seed production. The different treatments given the heads covered with tarlatan were to determine whether fertilization could be

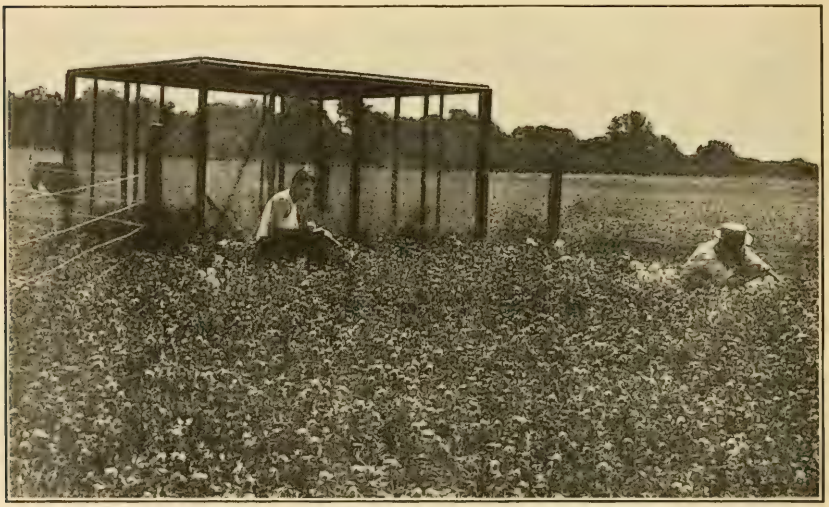

FIG. 3.—A screen cage (in the background) in which bumblebees were confined. Hand-pollination work is in progress in the foreground.

produced by any method of artificially manipulating clover flowers from which insects were excluded. For this work, plants were selected bearing at least eight heads which would come into bloom at approximately the same time. These plants were taken at random and each marked with a stake, as shown in figure 5. The heads on each plant were labeled from A to H, inclusive, and treated as shown in Table I.

These experiments were conducted in Iowa, in 1911 on 50 plants at Ames, and 25 at Altoona, and in 1912 on 70 plants at Ames.

Table II gives the results obtained on 25 representative plants, selected from the entire number, and also the average seed yield per head of the entire 145 plants experimented with in 1911 and 1912.

TABLE I.—*Treatment of clover blossoms in the artificial manipulation experiments.*

Head.	Heads not covered with tarlatan.	Heads covered with tarlatan.
A	Check.	
B	Entire head rolled between thumb and finger.	
C	Keel of each flower sprung with a toothpick, care being taken to rub the pollen on each stigma. (A separate toothpick was used for each head.)	
D	Tapped several times with coarse toothbrush.	
E		Check.
F		Worked same as B.
G		Worked same as C.
H		Worked same as D.

TABLE II.—*Effect of different types of artificial manipulation upon the seed production of red-clover plants treated in 1911 and 1912.*

25 SELECTED REPRESENTATIVE PLANTS.

Location, year, and designation of plants.	Number of seeds produced per head.							
	Heads not covered with tarlatan.				Heads covered with tarlatan.			
	A	B	C	D	E	F	G	H
Ames, 1911:								
No. 1	33	26	30	47	0	0	0	0
No. 2	40	46	39	26	0	0	0	0
No. 3	46	0	31	35	0	0	0	0
No. 4	40	69	20	36	0	0	0	1
No. 5	52	47	13	68	0	0	0	0
No. 6	54	18	52	56	2	0	0	1
No. 7	90	33	43	69	1	0	0	0
No. 8	44	57	62	40	0	1	0	0
No. 9	47	26	16	28	0	0	0	0
No. 10	82	55	59	65	0	0	3	0
Average	52.8	37.7	36.5	47.0	0.3	.1	.3	.2
Altoona, 1911:								
No. 11	26	13	24	5	0	1	0	0
No. 12	10	16	12	38	0	0	0	1
No. 13	29	12	4	7	0	0	1	0
No. 14	9	6	11	14	0	0	0	0
No. 15	22	16	28	0	0	0	0	0
Average	19.2	12.6	15.8	12.8	0	.2	.2	.2
Ames, 1912:								
No. 16	20	3	25	15	0	0	0	0
No. 17	33	31	24	36	0	0	0	0
No. 18	39	45	24	43	0	2	0	0
No. 19	20	40	28	21	0	0	0	0
No. 20	44	40	24	28	0	0	0	0
No. 21	52	61	51	52	0	0	1	0
No. 22	37	28	33	35	0	0	0	0
No. 23	40	35	40	21	0	0	0	0
No. 24	42	28	26	43	0	0	0	0
No. 25	15	5	6	12	0	0	0	1
Average	34.2	31.6	28.1	30.6	0	.2	.1	.1

SUMMARY OF AVERAGE RESULTS FOR THE ENTIRE 145 PLANTS.

Ames, 1911: 50 plants	44.3	38.8	35.1	42.3	0.08	0.16	0.16	0.22
Altoona, 1911: 25 plants	16.0	9.4	20.3	18.0	.28	.48	.24	.36
Ames, 1912: 70 plants	48.9	42.5	41.3	41.5	.1	.1	.14	.15
Average, 145 plants	41.6	35.5	35.5	37.7	.12	.18	.16	.21

The average seed yields given in the first part of Table II should be compared with the average seed production shown in the sum-

mary of the same table. The 25 plants listed separately were selected to represent all the plants upon which this experiment had been conducted in 1911 and 1912. While the average results shown by the selected plants vary somewhat from the results of all the plants, still they are representative of the plants as a whole. It will be noted that the seed production of the uncovered heads varies considerably on the same plant, so that final results must be taken from the average of treated heads on a number of plants rather than on a few plants. For this reason the results given in the summary more nearly represent true conditions than those given in the first part of Table II.

From the results obtained on the heads not covered with tarlatan it will be seen that artificial manipulation was detrimental to seed production, since the average yield of the check is higher than that

FIG. 4.—Heads of red clover covered with tarlatan to prevent pollination by insects.

of any treated series. This is undoubtedly due to the fact that the flowers were somewhat mutilated during the operation. Very little seed was obtained from the heads which were kept under cover and artificially manipulated. The few seeds obtained were probably the result of cross-pollination by bumblebees when the tarlatan cloth had been pushed against the heads by rain or had been cut by grasshoppers. Rains would wash the starch from the tarlatan, thus permitting it to fall against the clover heads and allowing the flowers to protrude. This was avoided by either straightening out the cloth after it had dried or re-covering the heads. A few flowers on some heads, however, were exposed to the action of insects for a very short time. In the work which was conducted at Altoona, Iowa the grasshoppers were so bad that some heads had to be re-covered

as often as three times a day. Many uncovered heads were partly destroyed by the grasshoppers, and this undoubtedly accounts for the small seed yield of the uncovered heads, since bumblebees were plentiful.

HEADS COVERED AND NOT POLLINATED.

Another experiment was conducted in order to determine whether clover heads kept covered during their entire blooming period and not pollinated could set seed.

Plants having at least six heads which would come into bloom at approximately the same time were selected for this work. Fifty

FIG. 5.—General view of the field in which the clover work was conducted in 1912 at Ames, Iowa. The stakes represent plants selected for individual pollination work. The cages in the background were used to test the efficiency of different insects as pollinators of red clover.

plants at Ames and 25 at Altoona were selected in 1911 and 27 plants at Ames in 1912. The average seed yields per head are shown in Table III.

TABLE III.—*Average seed yields of clover heads which were covered with tarlatan and not pollinated.*

Location, year, and number of plants.	Heads covered with tarlatan.					
	A	B	C	D	E	F
Ames, 1911: 50 plants	0.1	0.11	0.15	0	0.16	0
Altoona, 1911: 25 plants	.16	.4	.35	.04	.26	.2
Ames, 1912: 27 plants	0	.1	0	0	.02	0
Average, 102 plants	.08	.17	.15	.009	.14	.04

To the results presented in Table III may be added the results given in column E of the summary of Table II, where 145 heads were

covered, not treated, and used as checks in those experiments. From the 757 heads covered and not treated in 1911 and 1912 an average of 0.1 seed per head was obtained. The relatively high average obtained at Altoona in 1911 may undoubtedly be accounted for by grasshoppers mutilating the tarlatan which was used to cover the heads. On this account heads were occasionally exposed to the action of insects for a short time.

Since no more seed was produced by these heads than may be accounted for by insects working on the flowers when they were occasionally exposed for a short time on account of rains or grasshoppers, we may say that clover flowers must be pollinated by some agency before any seed is produced.

EFFECT OF SELF-POLLINATION.

Another experiment was conducted in which the clover heads were covered with tarlatan before any flowers opened and were kept covered, except while being worked, until mature. As soon as the flowers came into bloom they were self-pollinated by springing the keels of the flowers with toothpicks, care being taken to rub pollen upon each stigma. A separate toothpick was used for each head. In 1911, 125 heads were self-pollinated and 170 heads in 1912. An average of 0.16 seed per head was obtained in 1911 and an average of 0.09 seed per head in 1912.

The results of this experiment show, as have previous experiments, that red-clover flowers must be cross-pollinated in order to set seed on a commercial basis. The amount of seed obtained is so small that it was probably the result of bees working through the tarlatan, although the cytological work reported upon in this bulletin shows that it is possible to have an occasional seed produced from self-pollination.

SEED PRODUCTION OF HEADS UNDER ORDINARY FIELD CONDITIONS.

As a field check on the preceding experiments a number of heads were tagged in 1911 and 1912 and neither covered nor artificially pollinated. These heads were labeled in different parts of the field and Table IV shows the number of heads in each group and the average seed yield per head.

TABLE IV.—*Average seed yield of clover heads not covered or artificially pollinated.*

Location and year.	Number of heads collected.	Average number of seeds per head.
Ames, 1911	300	50.1
Do	532	55.4
Do	470	50.9
Altoona, 1911	150	43.6
Ames, 1912	65	53.4

The results given in Table IV are somewhat higher than the average seed yield of the uncovered check of the experiments summarized in Table II. It may be that the close proximity of the checks given in Table II to heads covered with tarlatan kept bees from making as many visits to those heads as they would otherwise have made.

FLOWERS POLLINATED WITH POLLEN FROM ANOTHER HEAD ON THE SAME PRIMARY BRANCH.

Since the amount of seed obtained in 1911 from self-pollinated heads under cover was so small that it could be accounted for by bees working through the tarlatan, it was not deemed necessary to emasculate the flowers for cross-pollination work in 1912. With this in view a series of heads was covered before any of the flowers came into bloom and later pollinated with pollen from another head on the same primary branch, 20 flowers on each of 11 heads being pollinated in this manner. Not a single seed was produced.

FLOWERS POLLINATED WITH POLLEN FROM A HEAD ON A DIFFERENT PRIMARY BRANCH OF THE SAME PLANT.

Another experiment similar to the preceding one was conducted, except that the pollen was taken from heads on different primary branches of the same plant, 20 flowers on each of 10 heads being pollinated in this manner. One seed was produced.

CROSS-POLLINATION EXPERIMENTS.

Alternately with the above two experiments 20 flowers on each of 13 heads were pollinated with pollen from a separate plant. An average of 14.3 seeds per head was obtained.

The results obtained in the last three experiments, as well as with all preceding ones, show that clover is practically self-sterile and that pollen must come from a separate plant in order to effect fertilization.

BUMBLEBEES AS CROSS-POLLINATORS OF RED CLOVER.

In view of the consensus of opinion that the bumblebee is responsible for the cross-pollination of red-clover flowers, and since no investigator, so far recalled, has denied its ability to do this, it was deemed desirable to study the relative efficiency of the bumblebee as a cross-pollinator of this plant.

For this work a cage 12 feet square and 6 feet high, made of wire screen having 14 meshes to the linear inch, was erected shortly after the first crop of clover had been cut. As soon as the second crop started to bloom bumblebees were caught with an insect net and placed in the cage. It was soon found that bees would live about three days when confined in the cage and that six bees in confinement would visit approximately as many flowers as one bee would have visited had it worked nearly all the time. With this in mind, two bumblebees were placed in the cage each forenoon until all the clover

heads were mature. An area 4 feet square was marked off in this cage as soon as the clover was mature. From this area all heads were collected, kept separate, and thrashed by hand. Of the 311 heads collected from this area an average of 30.4 seeds per head was obtained.

Repeated field observations in Iowa in 1911 and 1912 showed that bumblebees were actively engaged in collecting nectar from eight to nine hours a day. Little work was done by them before the dew had entirely disappeared from the foliage and flowers or after 6 o'clock in the evening. Observations showed that bumblebees are able to pollinate 30 to 35 flowers a minute. However, they seldom visit more than eight to ten on a single head at one time.

These results agree closely with those of Pammel and King (32), who state that bumblebees pollinate on an average 30 flowers a minute, and Smith, according to Beal (3), who estimates from counts that old bees will visit 35 flowers a minute and young bees seldom more than eight.

HONEYBEES AS CROSS-POLLINATORS OF RED CLOVER.

The ability of the honeybee to cross-pollinate red clover has been discussed by scientific investigators and beekeepers for some time. Those who do not believe that the honeybee is able to pollinate red clover base their statements for the most part on the fact that the proboscis of the honeybee is not long enough to reach the nectar located at the base of the staminal tube. Some investigators and bee men state that some strains of the Italian race of honeybees are able to obtain some nectar from red-clover flowers, while other investigators say that honeybees collect pollen from red-clover flowers and thereby cross-pollinate them.

According to Knuth (22, v. 2, p. 289) the proboscis of the honeybee is 6 mm. in length, which is 3.6 mm. shorter than the average length of the corolla tubes of first-crop red-clover flowers. Honeybees may be able at times to obtain some nectar from the sides of the staminal tubes of red-clover flowers when a large amount is secreted or when the flowers are not in an upright position. Knuth (22, v. 2, p. 289) observes that *Bombus terrestris*, a species of bumblebee found in Europe, pierces the tubes of clover flowers and that honeybees later obtain nectar through these slits. *Bombus terrestris* has a proboscis from 7 to 9 mm. in length. While working on the experiments reported upon in this bulletin several corolla tubes were observed which had been slit at the base, but it can not be stated that these slits were made by bees. Schneck (34) states that the Virginia carpenter bee (*Xylocopa virginica*) slits the lower end of the corolla tubes of red-clover flowers and that he has observed honeybees obtaining nectar through the slits.

In order to determine the efficiency of the honeybee as a cross-pollinator of red clover, a cage 12 feet square and 6 feet high, made of galvanized-wire screen having 4 meshes to the linear inch, was erected in the same field as the bumblebee cage. It was previously determined that a mesh of this size would permit a honeybee, or any insect smaller than a honeybee, to pass through, but would not permit bumblebees to do so. Two weeks before the clover came into bloom a small colony of honeybees was placed in one corner of this cage (fig. 6). The bees soon learned to pass through the screen. By the time the clover began to bloom the bees had become accustomed to the cage, and while most of them worked on flowers outside, some could always be seen at work on the clover within the cage. Bees

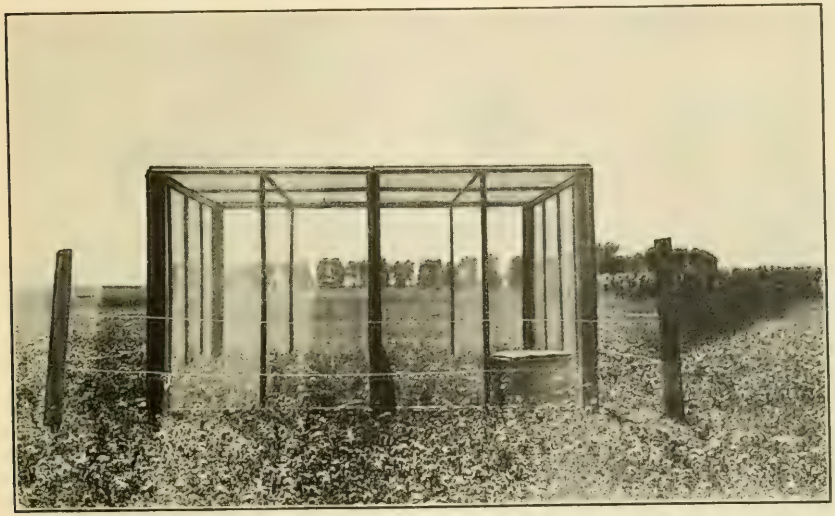

FIG. 6.—A screen cage in which a hive of honeybees was placed, in order to determine the efficiency of these insects as pollinators of red clover.

working on the clover within the cage were observed to collect pollen from the flowers and carry it to the hive.

As soon as all the flowers in the cage were mature, an area 4 feet square was measured off and all heads within this area were collected, kept separate, and thrashed by hand. Of the 623 heads collected from this area an average of 37.2 seeds per head was obtained.

The higher yield of seed obtained in the honeybee cage than in the bumblebee cage may be attributed, at least in part, to the larger number of bees which had access to this clover. However, the ratio of honeybees to bumblebees was no greater in the cages than in the clover fields in the vicinity of Ames in 1911.

In 1911 the precipitation at Ames was 2.48, 3.83, and 0.39 inches below normal for June, July, and August, respectively. When the

clover was in bloom very few nectar-producing plants were to be found. Whether the honeybee would work on red clover to this extent in a year of normal rainfall when the number of other nectar-producing plants is larger is problematical, but our observations and results show that the honeybee is able to spring the keels of red-clover flowers and thereby cross-pollinate them.

MECHANICAL CROSS-POLLINATORS OF RED CLOVER.

A machine so constructed that a platform of brushes could be made to strike clover heads with a vertical stroke was placed on the market under the name of a clover cross-pollinizing machine (fig. 7). This machine received some favorable comment. In view of this fact, a number of experiments were outlined to test its efficiency and also

FIG. 7.—Clover cross-pollinizing machine.

to test the efficiency of different types of hand-operated brushes as mechanical cross-pollinators of red clover. Some plats of red clover were treated with various types of brushes at different times of day, while other plats were treated when the clover heads were in different stages of bloom. The direction of the strokes with the brushes was also varied in order to see whether this would have any effect on the yield of seed.

MACHINE POLLINATION EXPERIMENTS.

In order to determine the efficiency of a clover cross-pollenizing machine several experiments were performed at Ames in 1911. Machines similar to the one used were offered for sale on the market at the time these experiments were being conducted.

This machine was so constructed as to give vertical strokes with a brush 4 feet wide and 6 feet long. The brushes could be removed with little trouble and replaced with others of a different type. Two types of brushes were used in these experiments. Brush No. 1 was composed of palmetto fiber, the bristles of which were rather stiff and of a dark reddish-brown color. Brush No. 2 was composed of rice-root fiber of light color, somewhat more flexible than the palmetto fiber. The plats treated were 12 feet wide and 100 feet long.

The number of heads collected from each plat represents the number contained within two hooped areas. These hooped areas were obtained by sailing a hoop into the air so that it would light on a particular plat. Since each plat contained a uniform stand it was thought that by this method heads would be selected which would be representative of the entire plat. Table V shows the results obtained in these experiments.

TABLE V.—*Average seed yield of red clover on plats which were given various treatments with a cross-pollinizing machine.*

No.	Treatments.					Heads collected.	Seeds per head.
	Brush used.	Times gone over.	Time between.	Time of day.	Number of days given.		
			Days.				
Plat 1	No. 1	1	1	A. M.	7	460	63.3
Plat 2	No. 1	2	1	A. M.	7	424	59.4
Plat 3	No. 1	1	3	A. M.	4	499	61.2
Plat 4, check						490	65.1
Plat 5	No. 1	2	3	A. M.	4	414	59.2
Plat 6	No. 1	3	3	A. M.	4	458	70.3
Plat 7, check						582	66.7
Plat 8	No. 2	3	3	A. M.	4	527	70.9
Plat 9	No. 1	3	3	P. M.	4	487	64.7
Plat 10, check						655	64.0
Plat 11	No. 2	3	3	P. M.	4	492	67.4

It will be seen from the above experiments that the treatments with brush No. 1 decreased the yield 1.9 seeds per head, while the plats treated with brush No. 2 gave an increased yield of 3.9 seeds per head over the average of the check plats.

EFFICIENCY OF DIFFERENT HAND-OPERATED BRUSHES AS MECHANICAL CROSS-POLLINATORS OF RED CLOVER.

In order to test the efficiency of hand-operated brushes as a means of mechanical pollination of red clover, eight pairs having different types of bristles were used. The different pairs were labeled A, B, C, etc., for convenience in reference. Following is a brief description of the different pairs of brushes used:

(A) Rice-root fiber, somewhat stiff; bristles about 2 inches long and nearly erect.

(B) Rice-root fiber, similar to A, but with bristles 3 inches long, somewhat coarser and more spreading.

(C) Tampico fiber, finer but stiffer than the rice-root fiber in brushes A and B.

(D) Wire-bristle hairbrushes.

(E) Indian palmetto fiber, coarser than any of the others, but somewhat brittle. A portion of this brush was modified by cutting out sections of the bristles to make a more uneven surface.

(F) Ordinary bristle hairbrushes.

(G) Same as brush E, but with about half of the bristles clipped out.

(H) Same as brush C, but with about half of the bristles clipped out.

The idea of utilizing pairs of brushes with different types of fibers was to determine, if possible, if any of them would give sufficient promise to warrant an application of the particular type of brush to a mechanically operated machine that would imitate the action of the small brush when operated by hand. For this reason no brushes were used which could not be duplicated on a machine for operation on a field scale.

The work with the hand-operated brushes was done principally at La Fayette, Ind., and Ames, Iowa. In some experiments the heads were manipulated with the brushes at different times of day, while in other experiments different numbers of treatments were given the heads at varying intervals. The direction from which the heads were struck also varied in certain experiments, some being given vertical strokes and others lateral strokes. It was thought that cross-pollination might be brought about by the vertical stroke, which apparently would enable some of the brush bristles to spring the keels and convey pollen from one flower to another. It was also thought that if the flowers were self-fertile the lateral strokes would accomplish this. The representative tables that follow indicate the principal features brought out by this series of experiments.

RELATIVE EFFICIENCY OF BRUSHES WHEN THE CLOVER HEADS WERE STRUCK HORIZONTALLY.

In a clover field 2½ miles east of La Fayette, Ind., 26 square-rod plats were laid off for this experiment in 1911 (Table VI). All of the heads in bloom at the time the plats were marked off were removed.

Plat 1 was left as a check, no brushes being used on it. Plat 2 was worked with brushes A, one brush being taken in each hand and the blossoms struck between the brushes by a quick movement of the wrists. When in full bloom the heads received one treatment, the operator going only in one direction across the plat. Brushes B, C, D, E, and F were used on plats 3, 4, 5, 6, and 7, respectively, in the same manner as for brushes A on plat 2. Plat 8 was treated by going one way across it at right angles to the first way, thus giving each blossom two treatments, the strokes of the two treatments thus being at right angles to each other. Brushes A were used. Plats 9, 10, 11, 12, and 13 each received treatment similar to plat 8, but with brushes B, C, D, E, and F, respectively. Plats 14 and 15 were left as checks. Plats 16, 17, 18, 19, 20, and 21 each received two treatments with

brushes B, C, E, B, C, and E, respectively, in the same manner as plat 8.

Two days later plats 16, 17, 18, 19, 20, and 21 each received two more treatments with brushes B, C, E, B, C, and E, respectively, and three days later plats 19, 20, and 21 each received an additional two treatments with brushes B, C, and E.

Plats 22 to 26, inclusive, were square-rod plats selected at intervals in the field immediately surrounding the portion laid off with the regular plats. These were designed to give a large number of check plats to show the variation in the field under ordinary conditions.

At harvest time 500 heads were picked at random from each of the 26 plats. These heads were hulled and the average seed production for the 500 heads is given in Table VI.

TABLE VI.—*Average seed yield per head obtained when clover heads were struck horizontally with different types of brushes and at different intervals at La Fayette, Ind., in 1911.*

No.	Strokes per treatment.	Brush used.	Date of treatment.	Heads collected.	Seeds per head.
Plat 1, check				500	58.2
Plat 2	1	A	August 2	500	46.0
Plat 3	1	B	do	500	67.1
Plat 4	1	C	do	500	50.1
Plat 5	1	D	do	500	27.0
Plat 6	1	E	do	500	39.4
Plat 7	1	F	do	500	34.7
Plat 8	2	A	do	500	44.0
Plat 9	2	B	do	500	35.4
Plat 10	2	C	do	500	59.4
Plat 11	2	D	do	500	27.2
Plat 12	2	E	do	500	34.7
Plat 13	2	F	do	500	59.9
Plat 14, check				500	49.3
Plat 15, check				500	57.0
Plat 16	2	B	August 2, 4	500	59.9
Plat 17	2	C	do	500	26.9
Plat 18	2	E	do	500	64.4
Plat 19	2	B	August 2, 4, 7	500	35.4
Plat 20	2	C	do	500	32.0
Plat 21	2	E	do	500	22.5
Plat 22, check				500	28.6
Plat 23, check				500	59.0
Plat 24, check				500	49.5
Plat 25, check				500	43.2
Plat 26, check				500	31.6
Average of all checks				4,000	47.0

Table VI shows that only 6 plats averaged higher than the average of the checks, whereas 12 plats averaged lower than the average of the checks. A variation almost as wide is shown in the 8 check plats as in the 18 treated plats, yet the results show that, as a whole, the brushes injured the seed production. The low results on the plats treated with brush D, the wire hair brush, were undoubtedly due to the fact that the wire bristles penetrated and injured the flowers.

This brush work was duplicated on a small scale with brushes A, B, and E on a few heads left uncovered and on heads that were covered with tarlatan, near La Fayette, in the summer of 1913. The heads were worked once. The results are given in Table VII.

TABLE VII.—*Average seed yield of clover heads which were struck horizontally with different types of brushes and either protected or unprotected from insects at La Fayette, Ind., in 1913.*

Brush used.	Strokes per treatment.	Heads left uncovered.		Heads covered with tarlatan.	
		Heads worked.	Seeds per head.	Heads worked.	Seeds per head.
A	1	3	37.4	1	0
B	1	5	38.8	1	0
E	1	5	50.1	2	0
Check	1	3	60.8	1	0
Do	1	5	57.5	1	0
Do	1	5	60.1	2	0

Table VII shows that in each case of the heads left uncovered the check produced the most seed. As in Table VI, the brushes apparently reduced the seed production. It will be noted also that both the brush-treated and untreated heads produced no seed when protected from insect visitation.

Experiments to test the relative efficiency of horizontal strokes with the different brushes were also conducted at Ames, in the summer of 1911. Ten plats 4 feet square were used. These plats were marked off by placing stakes at the corners and connecting these stakes with heavy cord. Plats 1 to 8 were each given one treatment on each of three consecutive days, with brushes B, A, E, C, F, D, G, and H, respectively, and plats 9 and 10 were left as checks. Duplicate tests, using the same brushes, respectively, on plats 37 to 44 were also made. The results are shown in Table VIII.

TABLE VIII.—*Average seed yield per head obtained when clover heads were struck horizontally with different types of brushes at Ames, Iowa, in 1911.*

Brush used.	Strokes per treatment.	Treatments.	Original tests.			Duplicate tests.		
			Plat No.	Heads worked.	Seeds per head.	Plat No.	Heads worked.	Seeds per head.
B	1	3	1	464	44.8	37	438	44.9
A	1	3	2	476	41.1	38	515	43.2
E	1	3	3	457	42.6	39	490	30.1
C	1	3	4	322	46.2	40	485	42.5
F	1	3	5	440	43.9	41	470	47.5
D	1	3	6	435	36.2	42	445	33.0
G	1	3	7	320	45.1	43	460	39.9
H	1	3	8	430	41.0	44	432	42.9
Check			9	532	55.4	9	532	55.4
Do			10	470	50.9	10	470	50.9

Table VIII shows that in every case where brushes were used the seed production fell below the yield of the check plats.

From the results obtained in Tables VI, VII, and VIII it is concluded that at least horizontal strokes with the brushes in question reduced the seed production on account of the flowers being mutilated by the brushes.

RELATIVE EFFICIENCY OF BRUSHES WHEN THE CLOVER HEADS WERE STRUCK VERTICALLY.

In order to test the efficiency of brushes in promoting cross-pollination by carrying pollen from one plant to another on the bristles of the brushes, experiments were conducted at Ames, Iowa, in the summer of 1911 on plats 13 to 20 with different pairs of brushes. A vertical stroke on each of three mornings, three days apart, was given. The plats were 4 feet square. At maturity all heads from each plat were collected, kept separate, and later thrashed.

The experiments on plats 21 to 28 were the same in all respects, except that one treatment when the flowers were in early bloom, instead of three treatments, was given each plat. Plats 9 and 10 were used as checks. The results are presented in Table IX.

TABLE IX.—*Average seed yield per head obtained when clover heads were struck vertically with different types of brushes at Ames, Iowa, in 1911.*

Brush used.	Strokes per treatment.	Plats given three treatments.			Plats given a single treatment.		
		Plat No.	Heads worked.	Seeds per head.	Plat No.	Heads worked.	Seeds per head.
Check		9	532	55.4	9	532	55.4
Do		10	470	50.9	10	470	50.9
B	1	13	366	37.0	21	442	46.8
A	1	14	490	42.0	22	440	33.8
E	1	15	440	42.1	23	420	45.0
C	1	16	521	45.3	24	415	38.9
F	1	17	416	39.3	25	380	54.5
D	1	18	476	37.7	26	400	45.9
G	1	19	510	41.8	27	430	48.1
H	1	20	462	44.7	28	436	49.0

Table IX shows that with the exception of plat 25 the treatment considerably reduced the yield below that of the lowest yielding check plat. The results of the treated plats 13 to 20, inclusive, taken as a whole show a decrease of four seeds per head less than the yield of plats 21 to 28, inclusive. This may be accounted for by the fact that plats 13 to 20, inclusive, received two more treatments with the brushes than plats 21 to 28, inclusive, and were therefore subject to more injury from the bristles of the brushes. It will be seen from these experiments that the vertical strokes with the brushes proved no more efficient than the horizontal strokes in the production of seed.

RELATIVE EFFICIENCY OF BRUSHES WHEN PRESSED TOGETHER BELOW THE CLOVER HEADS AND PULLED UPWARD.

Experiments were conducted in the summer of 1911, at Ames, Iowa, to determine the efficiency of pressing a pair of brushes together below the clover heads and pulling them upward with considerable force, but still not enough to break off the heads. The plats were 4 by 4 feet in size. Pair A of the brushes was used. Three treatments

three days apart in the forenoon were given. The results are presented in Table X.

TABLE X.—*Average seed yield per head when brushes were pressed together below the clover heads and pulled upward, summer of 1911.*

No.	Heads worked.	Brush used.	Treatments.	Seeds per head.
Plat 9, check	532			55.4
Plat 10, check	470			50.9
Plat 11	432	A	3	39.2
Plat 12	460	A	3	40.9

Table X shows that this manner of treatment as well as the horizontal and vertical stroke treatments caused a decrease in the production of seed.

SUMMARY.

A study of the cytology of red-clover flowers shows that many of them contain infertile ovules. The percentage of infertile ovules is greater in the first crop than in the second crop. In the first crop many plants produce 100 per cent of infertile ovules, while in the second crop the percentage of infertility ranges from none to a high figure. The percentage of infertile ovules in red clover is probably correlated with moisture conditions.

The pollen grains of red clover are very sensitive to moisture. On account of this, there can be little effective pollination when the flowers are wet. Germination of the pollen grains takes place only within a limited range of variation in the water supply. It is probably true that the only function of the stigma is that of supplying the requisite amount of water to the pollen for germination.

The time between pollination and fertilization varies with the temperature of the atmosphere. The time between pollination and fertilization in July is approximately 18 hours, while in October it varies from 35 to 50 hours. An examination of 30 flowers which had been self-pollinated for 55 hours showed good germination on the stigmas but no fertilization. The pollen tubes made a slow growth and none exceeded 4 mm. in length. In flowers which had been self-pollinated for 90 hours one pollen tube attained a length of 7.5 mm., while the rest were 5 mm. or less in length. The pistils of red clover average about 12 mm. in length. Eggs were found to be disintegrating four days after the flowers opened.

The self-pollination and cross-pollination experiments which were conducted in the field checked up very closely with the results obtained from the cytological studies. The average yield of seed obtained on heads which were not pollinated and on heads which were self-pollinated in different ways was less than one-half of 1 per

cent. This small yield of seed may be accounted for by the occasional access of bees to these heads for a very short time, on account of rains or grasshoppers mutilating the tarlatan which was used to cover the heads.

The bumblebee is an efficient cross-pollinator of red clover. Bumblebees are able to pollinate from 30 to 35 flowers a minute.

The honeybee proved to be as efficient a cross-pollinator of red clover as the bumblebee in 1911. When the precipitation was considerably below normal in June, July, and August, 1911, and but few nectar-producing plants were to be found, honeybees collected large quantities of pollen from red clover. In order to collect pollen they must spring the keels of the flowers. In doing this they cross-pollinate the flowers.

A clover cross-pollinizing machine which was offered for sale on the market did not prove to be an efficient cross-pollinator of red clover.

The various types of hand-operated brushes which were used did not prove efficient as cross-pollinators of red clover. In nearly all cases where these brushes were used the seed yield was decreased instead of increased. This was undoubtedly due to the bristles of the brushes injuring the flowers, since the average seed yield of the plats which received three treatments with the brushes was lower than that of the plats which received but one treatment.

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